



Antibiotic Resistance Profiles of Fish Gut Bacteria: Implications for Aquaculture

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Abstract:

This study examines the antibiotic resistance profiles of bacteria isolated from the gut of different fish species. The objective is to understand the prevalence and patterns of antibiotic resistance, which has considerable implications for aquaculture practices. Bacterial isolates were tested for susceptibility to several antibiotics using standard disc diffusion method. The results indicate a high prevalence of resistance to frequently utilized antibiotics, particularly chloramphenicol, kanamycin, and gentamycin. These findings highlight the urgent need for improved antibiotic management practices in aquaculture to mitigate the spread of resistant bacteria and ensure the sustainability of aquaculture practices. Understanding the resistance profiles of fish gut bacteria is essential for developing effective strategies to manage antibiotic use and reduce the risk of resistance spreading to other environments.

Key words: Fish gut, Bacteria, Resistance, Chloramphenicol, Aquaculture

Introduction

Antibiotics are commonly used in aquaculture for prevention and treat the bacterial infections, and increase feed efficiency. In excess and improper use of these drugs have leading to the emergence of antibiotic-resistant bacterial strains and posing a high risk for aquaculture and public health (Alfiansah et al., 2020). The fish gut is a complex and dynamic microbial environment, it plays a role as a reservoir for resistant bacteria and their associated antibiotic resistance genes (ARGs). Antibiotic-resistant bacteria in the fish gut adversely effect on the health of farmed fish and represent significant dangers to the environment and public health by promoting the spreading of resistance in aquatic ecosystems (Biehler, K., et al. 2020).

Horizontal gene transfer processes, including the conjugation, transformation and transduction, facilitate the transfer of antibiotic resistance genes from fish gut bacteria to other bacteria those present in aquatic environment (Gao & Lin, 2019). This gene transfer mechanism facilitates the the spread of resistance to the pathogenic and non-pathogenic bacteria, including those that can infect humans and animals and posing the global issue of antimicrobial resistance (Lulijwa et al., 2020). Therefore, the overuse of antibiotics in aquaculture has been associated with the emergence of multidrug-resistant bacterial strains and making infections harder to treat with the available antibiotics (Cabello et al., 2020).

Understanding the antibiotic resistance profiles of fish gut bacteria is essential for developing effective strategies to manage antibiotic use in aquaculture and control the spread of resistance. This study aims to examine the prevalence and distribution of antibiotic-resistant bacteria in the guts of different fishes, identify the related antibiotic resistance and study the potential risk to their spread. (Xia et al., 2020; Miranda & Zemelman, 2019).

Materials and Methods

Sample Collection

The Fish samples were collected from different aquaculture sites following standardized sampling protocols (Alfiansah et al., 2020). Fish were euthanized according to ethical guidelines for animal care and use in aquaculture research (Costa et al., 2020). The fish gut was aseptically removed, and the gut contents were homogenized in sterile phosphate-buffered saline (PBS) under aseptic conditions. Homogenized samples were stored in refrigerator until further bacterial analysis (Xia et al., 2020).

Bacterial Isolation

Homogenized gut samples were serially diluted using sterile PBS and plated on a variety of selective and non-selective agar media, including nutrient agar for general bacterial growth, MacConkey agar for Gram-negative bacteria, Cetrimide agar for *Pseudomonas* species, and Mannitol salt agar for *Staphylococcus* species (Manna et al., 2021). The plates were incubated at 37°C for 24 hours to allow bacterial colony development. Distinct colonies were selected based on morphological characteristics such as size, shape and color and sub-cultured on freshly prepared agar plates to obtain pure isolates for further analysis (Gao & Lin, 2019).

Antibiotic Susceptibility Testing

The antibiotic susceptibility test of the isolated bacteria was determined using the Kirby-Bauer disk diffusion method by following the Clinical and Laboratory Standards Institute guidelines (Su et al., 2021). Bacterial suspensions were prepared in sterile saline to match the turbidity of the 0.5 McFarland standard. These suspensions were spread onto Mueller-Hinton agar plates using spreader. Antibiotic disks containing commonly used antibiotics in aquaculture—Chloramphenicol, Kanamycin, Gentamicin, Amoxicillin, Rifamycin, Ampicillin, Tetracycline, Sparfloxacin, and Erythromycin—were placed on the inoculated plates (Rigos & Smith, 2021). Then Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured and interpreted according to CLSI standards to determine the resistance profiles of isolated bacteria (Miranda & Zemelman, 2019).

Data Analysis

The zones were measured for each of the bacteria by using the disk diffusion method and noted according to the Clinical and Laboratory Standards Institute standard guidelines (Su et al., 2021). The prevalence of bacteria was calculated for each antibiotic by determining the percentage of isolates resistant to each Antibiotics. Statistical analysis was performed to assess significant differences in resistance patterns among the bacterial species (Lulijwa et al., 2020).

Results & Discussion

A total five different bacterial isolates were obtained from the gut samples of different fishes, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Proteus vulgaris*. The antibiotic susceptibility test showed considerable variation in the response of these isolates to the antibiotics tested, indicating the presence of different resistance patterns among fish gut bacteria.

Table 1: Antibiotic Resistance Profiles of Bacterial Isolates

Antibiotics	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Proteus</i>
Chloramphenicol	22	11	19	17	21
Kanamycin	19	14	RR	RR	19
Gentamycin	24	24	17	20	21
Amoxycillin	19	13	19	18	14
Rifamycin	RR	17	10	19	10
Ampicillin	RR	RR	14	RR	RR
Tetracycline	RR	19	RR	RR	RR
Sparfloxacin	23	13	RR	RR	RR
Erythromycin	10	10	RR	RR	RR

The highest resistance rate was observed against tetracycline and ampicillin and most of the bacterial isolates showed resistance to these antibiotics, the lowest resistance was observed for ciprofloxacin, showing its continued efficacy against some bacterial strains in aquaculture systems (Table 1; Fig. 1).

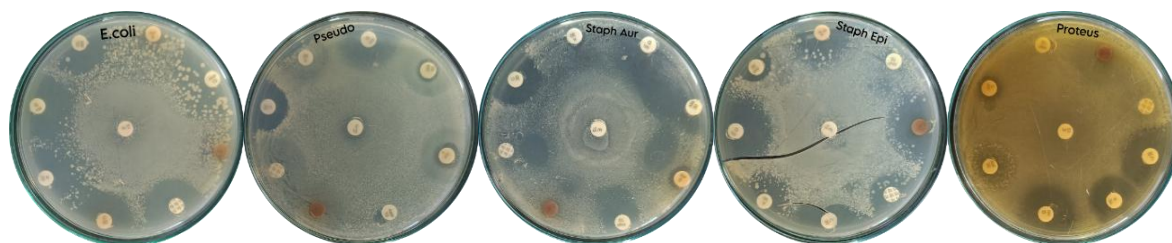


Fig. 1: Results of Antibiotic susceptibility using the disc diffusion method

Among the Gram-negative isolates, *E. coli* exhibited high susceptibility to gentamycin (24 mm), sparfloxacin (23 mm), chloramphenicol (22 mm), kanamycin (19 mm), and amoxycillin (19 mm). However, it was completely resistant to rifamycin, ampicillin, and tetracycline, while showing only weak sensitivity to erythromycin (10 mm). *Pseudomonas aeruginosa* demonstrated maximum sensitivity to gentamycin (24 mm), followed by tetracycline (19 mm), rifamycin (17 mm), kanamycin (14 mm), amoxycillin (13 mm), sparfloxacin (13 mm), chloramphenicol (11 mm), and erythromycin (10 mm). Complete resistance was observed only against ampicillin. *Proteus vulgaris* exhibited good susceptibility to chloramphenicol (21 mm), gentamycin (21 mm), and kanamycin (19 mm), whereas lower inhibition zones were recorded for amoxycillin (14 mm), rifamycin (10 mm), and complete resistance was observed against ampicillin,

tetracycline, sparfloracin, and erythromycin.

The Gram-positive isolates also displayed varying susceptibility patterns. *Staphylococcus aureus* was highly sensitive to chloramphenicol (19 mm), amoxycillin (19 mm), and gentamycin (17 mm), whereas moderate sensitivity was observed against ampicillin (14 mm), rifamycin (10 mm), and erythromycin-resistant isolates were common. Complete resistance was recorded against kanamycin, tetracycline, and sparfloracin. Similarly, *Staphylococcus epidermidis* showed maximum sensitivity to gentamycin (20 mm), rifamycin (19 mm), amoxycillin (18 mm), and chloramphenicol (17 mm), while complete resistance was observed against kanamycin, ampicillin, tetracycline, sparfloracin, and erythromycin.

Overall, the results revealed that ampicillin and tetracycline were the least effective antibiotics against most of the bacterial isolates, with four out of the five isolates showing complete resistance. In contrast, gentamycin demonstrated the highest antibacterial activity and was effective against all the isolates, producing inhibition zones ranging from 17 to 24 mm. Chloramphenicol also showed consistent activity against all isolates except for moderate inhibition in *P. aeruginosa*. These findings indicate the widespread occurrence of multidrug-resistant bacteria in the gut microbiota of fish and suggest that resistance to commonly used antibiotics has become well established in aquaculture environments.

The resistance observed against ampicillin and tetracycline is in agreement with recent reports describing these antibiotics as among the most frequently encountered resistance markers in aquaculture-associated bacteria. Their extensive use for disease prevention, treatment, and growth promotion has contributed to the selection of resistant bacterial populations in cultured fish and surrounding aquatic environments (Cabello et al., 2021; Lulijwa et al., 2020). β -lactam antibiotics such as ampicillin are particularly vulnerable to bacterial resistance because many aquatic bacteria produce β -lactamase enzymes that hydrolyze these compounds, thereby reducing their therapeutic effectiveness (Muziasari et al., 2021).

The occurrence of multidrug-resistant bacteria in the fish gut is of significant concern because these microorganisms may serve as reservoirs of antibiotic resistance genes (ARGs). Through horizontal gene transfer, these genes can spread to other environmental bacteria and opportunistic pathogens, increasing the risk of antimicrobial resistance throughout the aquatic ecosystem (Yang et al., 2023). Fish farming environments often receive continuous inputs of antimicrobial agents through medicated feed and contaminated water, creating selective pressure that favors the persistence and dissemination of resistant microorganisms (Schar et al., 2021).

The presence of resistant bacteria in edible fish also has implications for public health. Fish carrying multidrug-resistant microorganisms may introduce resistant bacteria into the food chain during harvesting, processing, or consumption if proper hygienic practices are not followed. Recent surveillance studies have emphasized that aquaculture should be recognized as an important component of the One Health framework because antimicrobial resistance can circulate among aquatic animals, humans, and the environment (World Health Organization [WHO], 2023; Aslam et al., 2021).

The findings of the present investigation support the need for responsible antibiotic use in aquaculture. Reducing unnecessary antibiotic application, implementing routine antimicrobial susceptibility testing before treatment, improving farm biosecurity, and adopting alternative disease management strategies such as probiotics, vaccination, and phytobiotics may help minimize the development and spread of antibiotic-resistant bacteria. Continuous monitoring of resistance profiles in fish-associated bacteria will also be essential for developing effective antimicrobial stewardship programs and ensuring sustainable aquaculture production.

Conclusion

This study highlights the widespread presence of antibiotic-resistant bacteria in the gut microbiota of aquaculture fish, showing resistance to antibiotics such as tetracycline and ampicillin. The results emphasize the urgent need for enhancing management protocols to control antibiotic usage in aquaculture and reduce the risk of further resistance development. Implementing sustainable approaches such as antibiotic rotation, probiotic administration, and improved farm hygiene can help mitigate the emergence and spread of resistant bacteria within aquaculture environments. Future research should focus on tracking antibiotic resistance patterns and exploring the potential transmission of antibiotic resistance genes (ARGs) from aquaculture systems to human pathogens through horizontal gene transfer.

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